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HYPOGLYCEMIC ACTIVITY OF SEVERAL SEAWEED EXTRACTS

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Summary

The hypoglycemic activity of several seaweed extracts on rabbits was studied. Ethanol extracts of *Laminaria ochroleuca*, *Saccorhiza polyschides* and *Fucus vesiculosus* were administered orally to normal animals and their effects on glycemia and triglyceridemia evaluated. Crude polysaccharides and protein solutions from *Himanthalia elongata* and *Codium tomentosum* were also assayed. Polysaccharides and proteins from *H. elongata* caused a significant reduction in blood glucose 8 h after intravenous administration. A dose of 5 mg/kg of crude polysaccharide lowered glycemia about 18% in normal rabbits and by about 50% in alloxan-diabetic animals, while the protein solution lowered glycemia in diabetic rabbits by about 30%.

Introduction

Several seaweeds have been reported to include hypoglycemic capacity among their biological activities. Certain *Sargassum*, *Cystoseira* (Phaeophyceae), *Corallina* and *Pterocladia* (Rhodophyceae) species have been shown to lower blood glucose and serum lipid levels (Bézanger-Beauquesne, 1982). *Laminaria* species which are employed against goitre in folk medicine also lower blood pressure and cholesterol levels (Hoppe, 1979), and *Fucus* has been used against goitre, obesity and diabetes (Lyon de Castro, 1981). Several algal macromolecule, polysaccharide and protein preparations have also been reported to be hypocholesterolemics and hypoglycemiants (Güven et al., 1979).

In this work, ethanolic extracts and crude polysaccharide and protein extracts from various seaweeds were assayed for their hypoglycemic properties in rabbits. The effects of the ethanol extracts on serum triglycerides were also studied.

Materials and methods

Seaweed collection and handling

The following marine algae were studied: *Laminaria ochroleuca* De la Pylaie (Laminariaceae), *Saccorhiza polyschides* (Lightfoot) Batters (Laminariaceae), *Fucus vesiculosus* L. (Fucaceae), *Himanthalia elongata* (L.) S.F. Gray (Himanthaliaceae), *Codium tomentosum* (Huds.) Stackhouse (Codiaceae).

In July – August 1986 and September 1987 *L. ochroleuca*, *F. vesiculosus*, *H. elongata* and *C. tomentosum* were hand picked at low tide on Porto Nadelas beach (La Coruña, Spain) and *S. polyschides* fronds were obtained in the Ria de Arosa area (Pontevedra, Spain). All specimens were authenticated by the Plant Biology Department of the University of Santiago de Compostela, Spain.

The fresh algal fronds were washed with tap water and cut in pieces. Part of this material was dried in a forced-air oven at 40 °C and stored.

Preparation of extracts

Ethanolic extracts. Fresh algae were stabilized with boiling 95% ethanol for 1 h. The resulting extracts (extract 1) were concentrated to dryness in vacuo at 40 °C, and the stabilized algae pieces were dried, powdered, stored and later extracted again with 95% ethanol to yield extract 2. Extract 2 was concentrated to a small volume in vacuo and added to the dry extract 1, and the resulting solution was evaporated to dryness in vacuo, dissolved in distilled water and stored at −20 °C until use. Percentage yields were as follows: *L. ochroleuca*, 21.2%; *S. polyschides*, 34.6%; *F. vesiculosus*, 24.1%.

Water-extractable polysaccharides. Dried seaweed samples (100g) were extracted for 4 h with 4 l of deionized water in a boiling water bath, with occasional stirring. The extracts were then filtered with suction through filter paper and 4 volumes of 95% ethanol were stirred into the filtrate at alkaline pH to precipitate polysaccharides (Su and Hassid, 1962). A 49.5% yield of polysaccharide was obtained from *H. elongata* and 2.2% from *C. tomentosum*. The sugar contents, as determined by the phenol-H_2SO_4 method (Dubois et al., 1956) and estimated as glucose, were 23% for *H. elongata* and 15% for *C. tomentosum*. Samples of polysaccharides were dissolved in deionized water and dialyzed against water in cellulose tubing (Sigma) for 48 h. The non-dialyzed portions were used to assay for hypoglycemic activity.

Protein extraction. Algal protein was isolated by the method of Güven and Güler (1979). Fresh algae were macerated with 2% sodium carbonate solution for 24 h. The extract was filtered and acidified and 3% barium chloride was added to the solution. The precipitate obtained was separated and extracted with 1% sodium carbonate and then filtered. The filtrate was neutralized and dialyzed as above. The non-dialyzed portion was used. The protein content was determined by the method of Lowry et al. (1951). Percentage yields by this technique were: *H. elongata*, 0.83%; *C. tomentosum*, 0.02%.

Biological assays

The animals used were normal male New Zealand rabbits weighing approximately 2 kg and fed mixed feeds (Saprogal) with free access to tap water. All assays were performed on animals fasted for 20 h but with water allowed ad libitum. All doses are expressed as g or mg equivalents of dried seaweed per kg of body weight, except for polysaccharides, which are expressed as mg of polysaccharide extract per kg of body weight. Ethanol extracts were administered intragastrically (10 ml/kg). Polysaccharide and protein extracts were administered intravenously (1 ml/kg) via the ear vein. All blood samples were withdrawn by puncture from the contralateral ear vein.

Effect on normoglycemic animals

Doses of 5, 10 and 20 g/kg of ethanol extracts from *L. ochroleuca*, *S. polyschides* and *F. vesiculosus* were administered to the fasted rabbits. Blood samples were taken immediately before administration (0 h) and +2, +4 and +6 h later. The doses assayed for *H. elongata* were 2.5, 5 and 10 mg/kg for polysaccharides and 100, 200 and 400 mg/kg of protein solution and for *C. tomentosum*, 5 and 10 mg/kg for polysaccharides and 500 and 900 mg/kg for protein solution. When polysaccharide and protein solutions were administered, blood samples were taken at 0, +1, +3, +6 and +8 h. The concentration of glucose in serum samples was measured in a Seralyzer reflectance photometer (Ames, Miles Laboratories) using Seralyzer reagent strips. Percentage variations of glycemia with respect to the initial (0 h) level was calculated (Lamela et al., 1985). Animals dosed with distilled water were used as controls.

Alloxan-diabetic rabbits

Chronically hyperglycemic rabbits were obtained by intravenous injection (in the ear vein) of 150 mg/kg of alloxan monohydrate (Sigma) dissolved in 0.9% saline (Akhtar and Ali, 1984). Seven days after administration, serum glucose levels of surviving rabbits (about 80%) were determined. Animals with fasting glycemia of 300 mg% or more were used. Polysaccharide and protein extracts from *H. elongata* were assayed. Doses of 5 mg/kg for polysaccharide and 200 mg/kg for protein solution were administered intravenously in the ear vein and blood samples were taken at 0, +1, +3, +6 and +8 h.

Effect of ethanol extracts on serum triglycerides

Ethanol extracts from *L. ochroleuca*, *S. polyschides* and *F. vesiculosus* were administered to normoglycemic animals as above, serum triglycerides were determined using a Seralyzer reflectance photometer (Ames, Miles Laboratories).

Statistical analysis

Data are given as means ± S.E. Differences between groups were

TABLE 1

EFFECT OF ETHANOLIC SEAWEED EXTRACTS ON SERUM GLUCOSE LEVELS OF NORMAL RABBITS

Treatment	Oral dosage (g/kg)	Initial glucose level (mg%)	Glycemia change (%)		
		0 h	+2 h	+4 h	+6 h
Control		125.5 ± 3.5	5.0 ± 1.7	3.5 ± 2.1	0.0 ± 2.0
L. ochroleuca	5	113.7 ± 4.6	5.2 ± 6.0	7.0 ± 5.9	6.7 ± 4.5
	10	115.8 ± 2.9	4.5 ± 3.9	−1.2 ± 3.2	4.2 ± 4.7
	20	128.0 ± 2.4	7.4 ± 4.7	−5.6 ± 4.1	−0.9 ± 2.2
S. polyschides	5	157.3 ± 5.5	−3.2 ± 2.6	−8.6 ± 4.9	−8.7 ± 5.6
	10	141.6 ± 5.1	−5.3 ± 4.7	−4.8 ± 4.4	−5.2 ± 3.9
	20	138.6 ± 6.4	1.1 ± 4.7	2.4 ± 6.3	2.2 ± 5.9
F. vesiculosus	5	120.5 ± 6.8	6.9 ± 8.7	14.1 ± 7.8	4.8 ± 7.8
	10	124.3 ± 2.1	−5.8 ± 1.6*	−9.6 ± 1.2*	3.0 ± 2.3
	20	127.5 ± 3.1	15.9 ± 6.2	6.1 ± 6.9	6.1 ± 2.2

Each value represents the mean ± S.E.M. of 6 rabbits.
*Significantly different from control, $P < 0.01$.

evaluated statistically using Student's t test (Tallarida and Murray, 1986). P values < 0.01 were taken to indicate significance.

Results and discussion

The yields of the ethanol extraction procedure were very high, especially the 34.6% of *S. polyschides*. This was probably due to the dilution of ethanol extractant during stabilization of the fresh seaweeds, which have a high water content, with the result that dissolved salts contribute to the final extract.

The low polysaccharide and protein contents of *C. tomentosum* (2.2% and 0.02% respectively) are also noteworthy. The purpose of dialyzing the polysaccharide and protein solutions was of course to remove salts and other molecules of low molecular weight.

Table 1 lists the effects of the ethanolic extracts of *L. ochroleuca*, *S. polyschides* and *F. vesiculosus* extracts on the glycemia of normal rabbits. Of all the assays performed, only oral administration of 10 g/kg of *F. vesicolosus* extract caused a statistically significant reduction of blood glucose, and even in this case the reduction was only slight (5.8% at +2 h and 9.6% at +4 h). Doubt is thrown on its true significance by the absence of any effect with 20 g/kg doses.

Table 2 lists the effects of the same ethanolic extracts on serum triglyceride levels. Oral administration of 20 g/kg of *L. ochroleuca* extract produced a statistically significant 20% reduction at +4 h, and triglyceride levels were still 18% below those of the controls at +6 h, although this difference was no longer statistically significant. *S. polyschides* extracts, on the other hand, increased serum triglyceride levels by a statistically significant 36% 6 h after administration of a 20 g/kg dose. *F. vesiculosus* had no effect at all on triglycerides at the dosages assayed.

Although the relationship between lipid and glucose metabolism suggest, a priori, that the glycemia and triglyceride results of Tables 1 and 2 ought to be related, they seem, in actual fact, to be quite independent of each other.

Table 3 shows that the results of assaying seaweed polysaccharides and protein solutions were more satisfactory, at least in the case of *H. elongata*. Intravenous administration of 5 mg/kg of crude *H. elongata* polysaccharides caused a significant 18% drop in the glycemia of normal animals at +8 h, and at 10 mg/kg a drop that was smaller (13.6%) but still significant. Analogous results were obtained by intravenous injection of crude *H. elongata* protein: doses of 200 and 400 mg/kg achieved respectively 17.7% and 18.2% reductions in glycemia at +8 h. In blood samples taken from randomly selected rabbits 24 h after injection of these dosages, glycemia had returned to its initial level (results not shown in Table 3). For some of the dosages assayed, smaller reductions in glycemia were already apparent sooner than 8 h after administration. Neither protein nor polysaccharide from *C. tomentosum* produced any significant change in glycemia by +8 h, though slight reductions were observed at +1 h after administration.

TABLE 2

EFFECT OF ETHANOLIC SEAWEED EXTRACTS ON SERUM TRIGLYCERIDES (TG)

Treatment	Oral dosage (g/kg)	Initial TG level (mg%)	TG change (%)	
		0 h	+4 h	+6 h
Control		61.0 ± 4.6	3.4 ± 4.7	−4.5 ± 5.5
L. ochroleuca	5	81.5 ± 4.7	5.2 ± 9.1	10.8 ± 7.7
	10	91.3 ± 3.0	−10.9 ± 2.7	−12.5 ± 7.1
	20	68.5 ± 4.6	−20.4 ± 2.3*	−18.4 ± 3.2
S. polyschides	5	97.0 ± 5.8	10.8 ± 8.3	8.3 ± 5.5
	20	52.7 ± 5.5	17.5 ± 7.0	31.7 ± 6.2*
F. vesiculosus	5	93.8 ± 12.0	0.0 ± 8.1	−3.0 ± 2.4
	10	59.2 ± 7.1	6.5 ± 2.6	6.3 ± 4.1
	20	57.2 ± 7.1	−0.9 ± 8.9	−7.7 ± 4.7

Values are mean ± S.E.M. (4−6 rabbits/group).
*Significantly different from control, $P < 0.01$.

TABLE 3

EFFECTS OF POLYSACCHARIDE AND PROTEIN EXTRACTS ON NORMOGLYCEMIC RABBITS

Treatment	Intravenous dosage (mg/kg)	Initial glucose level (mg%)	Glycemia change (%)			
		0 h	+1 h	+3 h	+6 h	+8 h
Control		140.2 ± 1.8	3.9 ± 1.8	−4.0 ± 1.8	−3.9 ± 1.5	−4.0 ± 1.9
Polysaccharides						
C. tomentosum	5	149.5 ± 1.4	−4.6 ± 2.0*	−5.9 ± 2.3	−7.8 ± 1.4	−8.4 ± 1.6
	10	138.3 ± 1.7	−2.2 ± 1.5	2.1 ± 1.5	−0.8 ± 2.5	1.2 ± 2.2
H. elongata	2.5	145.6 ± 3.2	−2.5 ± 2.5	5.2 ± 3.2	−12.5 ± 2.7	−9.6 ± 2.0
	5	139.4 ± 2.3	−6.6 ± 2.4*	−1.5 ± 1.5	−13.3 ± 0.7*	−18.0 ± 1.5*
	10	138.8 ± 1.7	0.8 ± 2.3	−0.3 ± 1.8	−10.5 ± 1.9	−13.6 ± 1.9*
Protein extract						
C. tomentosum	500	147.2 ± 3.9	−8.8 ± 2.0*	2.3 ± 1.7	−5.1 ± 3.9	8.2 ± 2.8
	900	149.5 ± 1.4	−7.1 ± 3.7*	2.3 ± 3.8	−8.9 ± 1.8	−9.3 ± 2.4
H. elongata	100	135.8 ± 1.7	−1.1 ± 1.3	2.7 ± 1.7	−4.4 ± 2.9	−11.6 ± 2.9
	200	135.6 ± 2.8	−3.3 ± 1.8*	−4.9 ± 1.6	−16.2 ± 2.8*	−17.7 ± 2.5*
	400	136.1 ± 3.4	−4.8 ± 3.4	−5.9 ± 2.7	−16.1 ± 2.6*	−18.2 ± 1.9*

Each value represents the mean ± S.E.M. of 6–10 rabbits.
*Significantly different from control, $P < 0.01$.

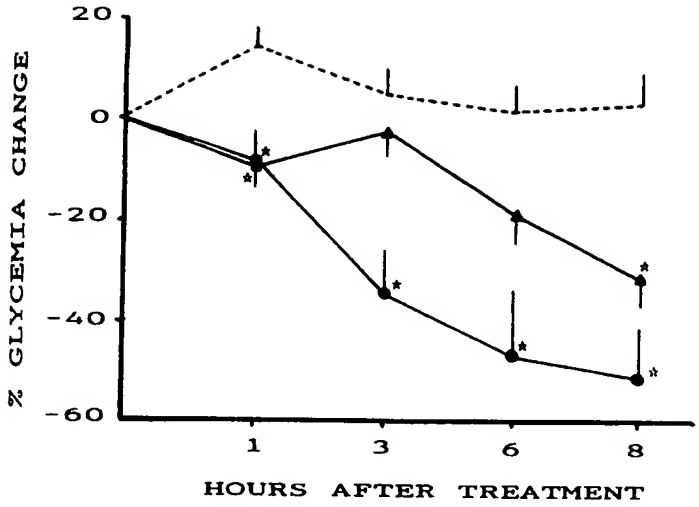

Fig. 1. Effects of *H. elongata* extracts on alloxan-diabetic rabbits. (– – –, Control; —▲—, 200 mg/kg protein extract; —●—, 5 mg/kg polysaccharide). Each point represents the mean ± S.E.M. of 5–9 rabbits. *Significantly different from control, $P < 0.01$.

In view of the evident hypoglycemiant effect of the *H. elongata* extracts on normoglycemic animals, 5 mg/kg doses of crude polysaccharide and 200 mg/kg of protein solution were assayed on rabbits with alloxan-induced diabetes whose initial fasting glycemia levels ranged from 304 to 572 mg%. Hypoglycemiant effects similar to those found in normal animals were observed, with +8 h reductions of 31.5% and 51.4% being caused by the protein and polysaccharide respectively (Fig. 1).

Pending further experimentation, the mechanism of the hypoglycemiant activity of the *H. elongata* extracts can only be conjectured (an effect on insulin secretion, for example, could be revealed by monitoring insulin levels after administration of the extracts). It seems likely, however, that the activity of the polysaccharide extract may have the same mechanism as various polysaccharides isolated from terrestrial plants (Tomoda et al., 1986), while that of the protein solution may be similar to that of the hypoglycemiant protein fraction isolated by Güven and Güler (1979) from the red alga *Pterocladia capillacea*. We are currently continuing our work on *H. elongata* with a view to answering these questions.

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